Workshop to Identify Critical Windows of Exposure for Children's Health: Cancer Work Group Summary

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We considered whether there are discrete windows of vulnerability in the development of cancer and which time periods may be of the greatest importance. Cancer was considered broadly, including cancers in childhood as well as adult cancers that may have an *in utero* or childhood origin. We concluded that there was evidence from animal and epidemiologic studies for causal relationships for preconceptional, *in utero*, and childhood exposures and cancer occurrence in children and adults. However, the evidence is incomplete and all relevant critical windows may not have been identified. The comprehensive evaluation of the relative importance of specific time windows of exposure is limited. Improvements in the design of epidemiologic studies and additional animal studies of mechanisms are warranted. *Key words*: adult, child, environmental, neoplasm. — *Environ Health Perspect* 108(suppl 3):595–597 (2000).
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Anderson et al. (*1*) raised a number of observations and issues on the human epidemiologic and animal evidence for windows of vulnerability in the development of cancer. This report summarizes these observations and issues considered by the cancer breakout group. We considered whether there are discrete windows of vulnerability in the development of cancer and, if so, which time periods are of the greatest importance. We discussed cancer in a broad context, with consideration of cancers in childhood as well as adult cancers that may have an *in utero* or childhood origin.

Priority Questions

The animal and human epidemiologic findings summarized by Anderson et al. (*1*) provide evidence for the association between exposures in specific windows and increased risks of cancer in childhood and adulthood. For example, some animal experiments using chemicals or radiation showed that exposure of the male animal before mating with an untreated female results in an excess of offspring with cancer (*1*). This finding has a number of implications that have generally not been fully appreciated. First, it shows that exposure to toxicants during the preconception period can result in cancer in the next generation. Second, the experiments indicate that paternal exposures can increase cancer risk in offspring (*1*). The area of male-mediated developmental toxicity remains controversial given the inconsistent evidence for association in epidemiologic studies of childhood cancer (*1,2*). Nonetheless, the relatively consistent animal evidence suggests that studies of adult cancer should consider the role of paternal exposures as well as maternal and *in utero* influences.

The evidence for the *in utero* period as a critical window is based in large part on the compelling results from the study of diethylstilbestrol and vaginal adenocarcinoma in young women (*3*). A number of early studies reported an association between ionizing radiation and leukemia in childhood (*4*). Finally, results from the study of Japanese atomic bomb survivors find an increased risk of breast and other cancers in adulthood after childhood exposure to high-dose radiation (*5*).

Thus, there appears to be strong evidence from animal and epidemiologic studies for causal relationships including exposure preconceptionally, *in utero*, and during childhood and cancer occurrence in children and adults. Nevertheless, Table 1 shows that even for the best-studied exposure (ionizing radiation), the evidence is incomplete for certain windows of exposure, in particular prenatal exposures and adult cancer. Therefore, all relevant critical windows may not have been delineated. Further, the comprehensive evaluation of the relative importance of specific time windows of exposure is limited by the lack of empirical evidence. The complexity of carcinogenesis and the lack of a general model will seriously inhibit a default prediction of critical window effects and direct incorporation into risk assessment models.

Issues

A major limitation in the available empirical evidence is the lack of a holistic pathogenetic model that encompasses childhood and adult cancers. Without such a unifying model, the theoretical basis of future epidemiologic and animal studies is missing. This is in contrast to other health outcomes such as birth defects, where one can use a well-described model of embryogenesis to make predictions about the likely effects of teratogens on specific structures at specific time periods. Of course, there are some general models of multistep carcinogenesis incorporating initiation and progression parameters that allow some predictions as to the potential early or late effects of agents. Experimental studies showed that there are a number of susceptibility factors at different stages of transplacental and neonatal carcinogenesis (e.g., target cells at risk, DNA repair capacity, metabolic detoxification, etc.) that are not really incorporated into the general multistep models in our current understanding of early and late effects of environmental exposures (*1*).

One model that has proven useful for considering childhood tumors is the two-stage model described by Knudson et al. (*6*). Knudson et al. (*6*) posited at least two events; the first is either a germline mutation (or other inactivating event) or somatic mutation, and the second is at least one somatic mutation. The authors used descriptive epidemiologic and genetic data to suggest that for several childhood cancers (e.g., Wilms tumor, neuroblastoma, and retinoblastoma), hereditary case diagnoses (those cases with a positive family history or those that inherited a *de novo* germline mutation or other features) would be at a younger

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Table 1. Critical time windows and ionizing radiation.[a]

Time window	Childhood cancer		Adult cancer	
	Human	Animal	Human	Animal
Preconception	Paternal: limited/suggestive	Paternal: none[b]	—	Sufficient for lung, lymphoid, skin, uterus Suggestive for liver, ovary, mammary
	Maternal: limited	Maternal: none[b]	—	
In utero	Maternal: abdominal X rays Sufficient for leukemia (limited relevance today)	Limited/suggestive	Limited	Sufficient for lymphoid, lung, liver, kidney, nervous system, lymphoid, ovary, pituitary, skin, sarcoma, uterus Suggestive for mammary gland, thyroid
Postnatal	Sufficient (leukemia, thyroid, other)	Suggestive for thymus and myeloid leukemia	High dose (Japanese atomic bomb) and several cancer types Therapeutic radiation and several cancers Risk of young onset breast cancer increased with exposure < 10 years old	Sufficient for lung, liver, kidney, lymphoid, uterus, Harderian gland Suggestive for ovary, skin, nervous system, mammary gland

[a]References to specific studies can be found in Anderson et al. (*1*). [b]Animal experiments usually do not produce childhood tumors based on age or histology.

age than for sporadic cases. This is because cases with germline mutations would be primed, with all cells of the offspring carrying the initial mutation and only a second somatic mutation would be required for tumor development. In these cases, compared with those requiring two somatic mutations (sporadic), the time to event would be shorter and would yield a younger age at diagnosis. Later molecular studies of tumor-suppressor genes lent support to the basic framework of the Knudson et al. (*6*) model. Thus, the model predicts that agents which result in a mutation (or epigenetic alteration) in the parental germline would result in childhood cancer cases with a younger age at diagnosis. This is most useful for thinking about paternal exposures where the expected mechanism or critical window would be a germline mutation. Although the possibility that fathers could bring home toxicants which could expose the pregnant mother and fetus cannot be ruled out, this is less likely than a direct effect on the male germ cells. This model can then be used to predict that paternal preconceptional exposure, if causally associated, would result in offspring with a younger age at diagnosis. Maternal preconceptional exposures cannot be ruled out, although they have not been as well studied. Thus, although indirect, this model provides some basis for considering a critical window for childhood cancers. Further, the Knudson et al. (*6*) model can be used to develop and test hypotheses related to adult cancers; exposures during the time periods around conception and pregnancy may provide the initial mutational event that, combined with additional events later in life, will increase the risk of cancer in adulthood. Similarly, a person with a germline alteration would be a greater risk of adult cancer with the presence of exposure-induced somatic alterations postnatally.

Another issue is that animal models, with some exceptions, do not permit direct analogy with childhood cancer. That is, in experimental animal studies, preconceptional, transplacental, and neonatal carcinogenesis is not comparable to childhood cancer in humans (*1*). There is not an analogous childhood in laboratory animals; therefore, most studies relate to adult human tumors on the basis of age of appearance and histology. Nonetheless, animal studies will continue to provide important data on the effects of exposure during various stages before, during, and after pregnancy. Further, these studies are important for their ability to identify susceptibility factors, to establish toxicokinetic models, and to provide mechanistic information (*1*).

Gaps in Knowledge

There are a number of gaps in knowledge on exposures in specific windows and increased risk of cancer [reviewed by Anderson et al. (*1*)]. Table 1 provides clear evidence for the sparseness of the data on ionizing radiation. In human studies there is a dire need for higher quality epidemiologic data on a range of exposures within and across time periods. These gaps exist because of a variety of limitations frequently encountered in epidemiologic studies, such as study size, exposure measurement, confounding, and other biases. Exposure assessment is a serious challenge in two respects. First, most recent studies for childhood cancer have been multicenter case–control studies. Obtaining valid exposure data from parents, often through self-report, remains a major concern. In adult cancer studies, obtaining extensive exposure data for the subject's own pregnancy and childhood or for their parents' preconceptional period is presently in most cases impossible. A basic problem with most epidemiologic studies is the difficulty in accounting for the interaction among exposures within and across time periods. Even for a single exposure, the ability to estimate meaningful effects of an exposure that occurs across multiple time periods can be limited. Currently, there is a great interest in the identification of genetic susceptibility factors in cancer and the role of gene–environment interactions in the development of childhood cancers. For example, a recent study of twins with acute lymphoblastic leukemia used polymerase chain reaction methods to detect a marker of chromosomal translocation and reported that this childhood leukemia is often initiated *in utero* but requires additional postnatal events (*7,8*). Epidemiologic studies are needed to evaluate the role of gene–environment interactions in these events. In addition, there are few studies of metabolic enzyme polymorphisms and exposures in relation to the risk of childhood cancers (*9*). A number of phase I enzymes (cytochrome P450s, for example) involved in the activation of a variety of carcinogens, medications, and other compounds, as well as phase II enzymes (e.g., glutathione *S*-transferases) that play a role in detoxication are possible candidates for investigation (*10*). Polymorphisms of genes involved in the DNA repair process are now available for evaluation (*11*). In addition, enzymes that metabolize nutrients, such as 5,10-methylenetetrahydrofolate reductase, are also of interest (*12*). It is not clear what the priority enzymes or receptors and related substrates or ligands should be or the relative contributions of paternal, maternal, or fetal genotypes. The identification of susceptibility

factors may strengthen previously moderate or weak associations or reveal new associations with exposures.

There are further gaps in the experimental animal data: Additional mechanistic information is needed. For example, studies of male exposure to chromium showed an increased incidence of cancer in offspring (*1*). However, the mechanism underlying these findings is not clear and an epigenetic mechanism may be of interest. Additional work on the molecular basis of epigenetic phenomena (e.g., methylation and imprinting) and the influence of toxicants on altering these events is warranted (*13*). The relationship of experimental studies with human data is not always direct; better linkage is required. Finally, much of the animal data relate to important exposures such as ionizing radiation and other known strong mutagens. However, there is a need to also study a broader range of exposures that may have a different structure–function relationship.

Recommendations

The gaps in knowledge suggest a number of recommendations for future research in this area. Epidemiologic studies can be improved in a number of ways. First, epidemiologists should be more opportunistic in investigating uniquely exposed cohorts. Cohorts such as those defined by occupation, medical treatment, geography, industrial accidents, or other scenarios offer the possibility to study the effects of higher dose, sometimes well-documented, exposures. For rarer cancers, case–control studies are often the design of choice. There is still the need for case–control studies that are population-based, larger, and with better exposure assessment methodology, although there have been some advances in this respect in recent years. New methods to obtain better occupational exposure data in community based case–control studies have been developed (*14*). The development, validation, and application of biomarkers of exposure and effect remain an active area of research (*15*). Where possible, studies should attempt to more precisely define potential dose–response relationships that will facilitate causal evaluation and risk assessment.

Advances in molecular biology provided the tools to investigate genetic susceptibility both in terms of somatic alterations of the tumor (e.g., *p53* tumor-suppressor gene mutations) and inherited alterations such as metabolizing enzyme polymorphisms. Epidemiologists should continue to attempt to integrate these tools into field studies. As another example, in some cases of Wilms tumor, a childhood kidney tumor, and retinoblastoma, a childhood eye tumor, the abnormal allele was inherited from the father (*16,17*). Epidemiologic studies could combine methods to identify the parent of origin for the mutation with parental exposure data to define more precisely the etiologic pathway and critical windows of exposure. The rapid development of molecular biology, dissection of the human genome, and databases will provide invaluable resources for these efforts.

Animal studies should continue to investigate specific mechanisms of cancer development with special attention to timing and windows of exposure. These studies can provide invaluable information for our understanding of the development of human cancers. Specific mechanisms such as epigenetic events are of great interest and animal studies offer the possibility to test hypotheses, particularly those related to the effects of endogenous and exogenous exposures. The ability to control the time window of exposure and genetic background should yield new information. These studies would be improved if they were guided, in part, by the findings from epidemiologic studies and explored a greater range of compounds on the basis of mechanism.

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